

Statistical Comparison of Three Methods for Determining Organic Peroxides

CONSTANTINE RICCIUTI, J. E. COLEMAN, and C. O. WILLITS

Eastern Utilization Research Branch, U. S. Department of Agriculture, Philadelphia 18, Pa.

The polarographic method for determining hydroperoxides was compared with the more commonly used Wheeler iodide and stannous chloride chemical methods. The Latin square experimental design and statistical analyses were used to determine the relative accuracy and precision of the results obtained. The three methods gave results which were not significantly different for high purity Tetralin hydroperoxide. For two hydroperoxide samples of lower purity and for three samples of autoxidized methyl oleate, the chemical methods gave values which were significantly higher than those by the polarographic method. With pure hydroperoxides the three methods apparently yield identical results, but with impure products the polarographic method may give more reliable values because it is more specific than the chemical procedures.

BARNARD and Hargrave (1) recently presented a critical review of chemical methods used for determining organic peroxides and reported that these methods have many sources of error. For example, the methods for ferrous ion oxidation are unreliable unless carried out carefully under controlled conditions; the commonly used iodide oxidation methods are subject to error because of the addition of iodine to olefinic double bonds and the effect of sample size.

Barnard and Hargrave developed a modified stannous chloride procedure, which when tested on peroxides and hydroperoxides of high purity (99 to 100%) gave theoretical values with an average standard deviation of only 0.32%.

The present investigators have used extensively a modification of the Wheeler (8) iodide methods for determining organic peroxides. Recently they developed a polarographic method, which, in contrast with chemical methods, distinguishes between peroxides and hydroperoxides and is specific for determining both. It was hoped that a statistical comparison between the stannous chloride, Wheeler iodide, and polarographic methods might explain some of the anomalous results which the present investigators had observed between the last two methods. They believed that a statistical appraisal might also show the relative precision and accuracy of the three methods.

To make a proper statistical evaluation of the three methods, not only should a variety of peroxidic samples and a sufficient number of replicates be included but consideration should be

given to the stability of the peroxide samples with respect to time and other conditions, such as exposure to air and room temperature during sampling. To make the statistical comparison of the three methods and to include the factors of stability, a series of statistically designed experiments based on a 3×3 Latin square arrangement (2, 4, 10) was conducted.

STATISTICAL DESIGN OF EXPERIMENT

The 3×3 Latin square was designed to include the three methods, three aliquots of each peroxidic material being analyzed, and the three different times at which the analyses were made. This randomized block arrangement was repeated for each of the six peroxidic materials included in the study. The Latin square arrangement is as follows:

Methods	Aliquots		
	1	2	3
I	M	W	F
II	W	F	M
III	F	M	W

where I, II, and III are the polarographic, iodide, and stannous chloride methods, respectively; 1, 2, and 3 are the three undiluted aliquots of a peroxidic sample; and M, W, and F are the days (Monday, Wednesday, and Friday) on which the analyses were made.

An analysis of variance as described by Snedecor (7) was then applied to the data obtained by the Latin square arrangement so that the effect of methods (polarographic *vs.* chemical and Wheeler iodide *vs.* stannous chloride), aliquots, times, interaction, and interaction within the three individual methods could be evaluated. In this experiment the three undiluted aliquots of each original material were transferred to separate containers and each of these aliquots were analyzed by the three methods on the three different days. The Snedecor *F* ratio, obtained by dividing in turn the mean squares for the methods, aliquots, times, etc., for each sample by the mean square for interaction, was compared to critical *F* values at the 5% level to determine whether the mean squares were statistically significant or not.

To determine if a difference exists between the values obtained by the three methods, the least significant difference was calculated. This consisted in comparing the mean values of the three sets of duplicates of the three undiluted aliquots for one method and one sample with the corresponding mean values ob-

tained by another method on the same sample. The corresponding duplicates in each set were averaged, and the differences (d), between the two averages obtained. These three differences were squared, giving d_1^2 , d_2^2 , and d_3^2 , which were then substituted in the following equation:

$$s_d^2 = \frac{1}{n-1} (d_1^2 + d_2^2 + d_3^2 - n\bar{d}^2)$$

where s_d^2 is the variance of the differences, n is the number of set of duplicates being compared (in this case, 3), and \bar{d}^2 is the square of the average difference. Then $\sqrt{s_d^2} = s_d$, the standard deviation of the differences, and $\frac{s_d}{\sqrt{n}} = s_{\bar{d}}$, the standard error of the differences between means, were obtained. The critical t value for the 2 degrees of freedom at the 5% level, 4.30, and $s_{\bar{d}}$ were used in calculating the limits of differences. A 5% risk was accepted on each comparison so that the combined comparison had a risk somewhere between 5 and 10%. These values were inserted in the following expression:

$$\left| \frac{\bar{d} - D}{s_{\bar{d}}} \right| \leq 4.30$$

where d = average of differences between each pair of means
 D = observed differences between means of two methods
 $s_{\bar{d}}$ = standard error of the differences between the three means

Transposing,

$$|\bar{d} - D| \leq 4.30 s_{\bar{d}}$$

and eliminating absolute values,

$$-4.30 s_{\bar{d}} \leq D \leq 4.30 s_{\bar{d}}$$

then adding \bar{d} to the term of the expression, we obtain

$$\bar{d} - 4.30 s_{\bar{d}} \leq D \leq \bar{d} + 4.30 s_{\bar{d}}$$

From this expression the least significant differences were calculated between the Wheeler iodide *vs.* the polarographic and between the Wheeler iodide *vs.* the stannous chloride methods.

EXPERIMENTAL

Preparation of Materials. Tetralin (1, 2, 3, 4-tetrahydronaphthalene) hydroperoxide, melting point 54–54.5° C., and cumene hydroperoxide, boiling point 65° C. at 0.1 mm. of mercury and $n_D^{20} = 1.5221$, were prepared as previously described (5). The autoxidized methyl oleate samples were prepared by autooxidation of methyl oleate followed by precipitation of unoxidized methyl oleate as the urea complex (8).

Procedures. POLAROGRAPHIC METHOD. The polarographic procedure has been described (9). A Sargent Model XXI polarograph was used to obtain the current-voltage curves; the electrolytic solution was 0.3M lithium chloride in 50 to 50 methanol-benzene; the electrolytic cell was a modified H-cell (9), and the capillary had m and t values of 3.116 mg. per second and 1.53 seconds, respectively, yielding a capillary constant of 2.29. The diffusion currents, i_d , were calculated from the wave heights of the hydroperoxide waves for the test samples with half-wave potentials of about –0.90 volt. The hydroperoxide content of the samples was calculated as follows:

$$\% \text{ hydroperoxide} = \frac{i_d \times 100}{C \times K}$$

where i_d = diffusion current, microamperes
 C = concentration of sample in H-cell, moles per liter

K = diffusion current constant of a standard hydroperoxide, microamperes per mole per liter

This K value (5.85 ± 0.05 for the capillary used) had been established (9) by analyzing highly purified samples of cumene Tetralin, and cyclohexene hydroperoxides.

STANNOUS CHLORIDE METHOD. The stannous chloride method has been described by Barnard and Hargrave (1). A weighed sample containing 0.75 to 1.0 meq. of peroxide is dissolved in acetic acid (10 ml.) in a 250-ml. Erlenmeyer flask, which is then evacuated to 20 mm. of mercury and filled with nitrogen. Fifteen milliliters of 0.1N stannous chloride solution are added from a pipet, and the flask is immediately re-evacuated and filled with nitrogen, the latter procedure being repeated twice. After standing for 1 hour at room temperature, a boiling solution consisting of 5 ml. of stock ferric solution, 1 gram of ammonium chloride, and 45 ml. water is added. The mixture is kept at 75° C. for 30 seconds and then rapidly cooled, and 10 ml. of phosphoric acid solution are added. The ferrous ion is titrated with 0.05N potassium dichromate solution and 6 drops of indicator solution; (0.25% solution of diphenylamine sulfonic acid in water); the end point is a sharp transition from green to violet. Blank determinations are carried out in a similar manner.

% peroxide =

$$\frac{(\text{blank} - \text{titer}) \times \text{dichromate normality} \times \text{mol. wt. of peroxide} \times 100}{2000 \times \text{weight of sample}}$$

IODIDE METHOD. The iodide method was a modified Wheeler method (6, 8). Twenty milliliters of 3 to 2 acetic acid-chloroform are introduced into a glass-stoppered 250-ml. iodine flask. A weighed sample adjusted to give the same final volume of reagent as the stannous chloride method is transferred to the flask and flushed with nitrogen. Two milliliters of freshly prepared 50% solution of potassium iodide in water are added, and the flask is again flushed quickly with nitrogen. After 15 minutes, 50 ml. of water are added, and the liberated iodine is immediately titrated with 0.1N sodium thiosulfate. The entire procedure is so arranged that the contents of the reaction flask are not in contact with any appreciable amount of dissolved or atmospheric oxygen at any time until the water is added.

Under these conditions, the liberated iodine rises to a maximum concentration in less than 15 minutes, and thereafter remains constant. Peroxide values obtained with reaction times up to 2 hours are no different from those obtained in 15 minutes.

% peroxide =

$$\frac{\text{ml. of thiosulfate} \times \text{normality} \times 0.008 \times 100}{\text{wt. of sample} \times \% \text{ peroxide oxygen in pure compound}}$$

Table I. Statistical Evaluation of Peroxide Methods by Latin Square Design

Aliquot	I Tetralin Hydroperoxide			II Cumene Hydroperoxide			III Impure Cumene Hydroperoxide		
	1	2	3	1	2	3	1	2	3
Methods									
Polarographic	M	W	F	F	M	W	M	W	F
Wheeler iodide	W	F	M	M	W	F	W	F	M
SnCl ₂	F	M	W	W	F	M	F	M	W
Polarographic	98.55	98.27	98.29	94.78	94.56	94.27	69.19	70.52	70.76
	98.68	100.30	98.97	94.31	94.57	95.43	68.70	71.72	69.65
Av.	98.62	99.29	98.63	94.55	94.57	94.85	68.95	71.12	70.21
Wheeler iodide	99.28	97.60	99.38	98.19	96.20	98.57	77.18	77.75	78.71
	98.87	99.18	100.00	99.70	96.20	98.87	77.28	75.38	79.47
Av.	99.08	98.39	99.69	98.95	96.20	98.72	77.23	76.57	79.09
SnCl ₂	98.43	92.48	97.69	98.56	99.10	96.85	78.79	78.12	78.59
	99.51	91.98	95.80	98.66	99.96	96.03	77.54	76.72	74.37
Av.	98.97	92.23	96.75	98.61	99.53	96.44	78.17	77.42	76.48
Aliquot	IV High Level Autoxidized Me Oleate (A)			V High Level Autoxidized Me Oleate (B)			VI High Level Autoxidized Me Oleate		
	1	2	3	1	2	3	1	2	3
Methods									
Polarographic	W	F	M	W	F	M	F	M	W
Wheeler iodide	F	M	W	F	M	W	M	W	F
SnCl ₂	M	W	F	M	W	F	W	F	M
Polarographic	73.37	72.83	73.06	69.54	69.71	69.53	20.84	20.75	20.89
	71.16	74.49	72.61	68.19	67.84	69.45	20.18	20.65	19.67
Av.	72.27	73.66	72.84	68.87	68.78	69.49	20.51	20.70	20.28
Wheeler iodide	77.61	77.00	78.23	78.44	80.00	79.26	21.97	22.38	21.76
	77.61	77.41	77.21	77.82	80.49	79.67	21.76	21.33	21.54
Av.	77.61	77.21	77.72	78.13	80.25	79.47	21.87	21.86	21.64
SnCl ₂	76.67	79.54	81.52	80.30	78.42	78.18	22.27	23.38	23.71
	72.96	78.52	82.54	81.41	78.56	78.49	23.57	23.77	23.21
Av.	74.82	79.03	82.03	80.86	78.49	78.34	22.92	23.58	23.46

Table II. Table of Mean Squares^a

Methods	DF	I	II	III	IV	V	VI
Polarographic vs. chemical	1	0.76	23.31	109.65	53.06	208.28	8.48
Wheeler iodide vs. SnCl ₂	1	1.27	0.08	0.11	1.86	0.00	3.47
Aliquots	2	0.21	0.43	0.18	10.73	0.03	0.08
Times	2	0.15	1.02	0.05	11.85	2.55	0.03
Interaction	2	2.97	3.47	3.33	4.69	0.91	0.09
Within polarographic	3	...	0.26	0.48	1.31	0.88	0.32
Within Wheeler iodide	3	...	0.40	1.03	0.20	0.13	0.20
Within SnCl ₂	3	...	0.24	3.55	2.47	0.23	0.34

^a Basis means of duplicates.

RESULTS AND DISCUSSION

Table I shows the six Latin squares for the six peroxide samples, including the duplicate peroxide values and their means expressed as percentage peroxide obtained on the respective days by the three peroxide methods. It is evident that high purity Tetralin hydroperoxide (I) is approximately 99% hydroperoxide, whereas the high purity cumene hydroperoxide (II) has a slightly lower hydroperoxide content. The impure cumene hydroperoxide (III), containing approximately 75% hydroperoxide, had originally been pure but had decomposed during a long storage period at room temperature. The two high-level autoxidized methyl oleates (IV, V) contained approximately 75% hydroperoxide and the low-level autoxidized methyl oleate (VI) had only 21% hydroperoxide. These samples were chosen because they represented a fairly wide range of hydroperoxide contents and provided samples containing different levels of decomposition impurities.

Table III. Limits of Differences between Means of Duplicates

Compound	Wheeler Iodide-Polarographic, %	Stannous Chloride-Wheeler Iodide, %
I Pure Tetralin hydroperoxide	$-0.2 \leq D \leq 1.8$	$-3.0 \leq D \leq 5.2$
II Pure cumene hydroperoxide	$-0.4 \leq D \leq 7.0$	$-2.2 \leq D \leq 6.2$
III Impure cumene hydroperoxide	$3.0 \leq D \leq 12.1$	$-1.0 \leq D \leq 3.9$
V High-level autoxidized methyl oleate A	$2.3 \leq D \leq 6.9$	$-0.2 \leq D \leq 6.1$
V High-level autoxidized methyl oleate B	$7.6 \leq D \leq 12.9$	$-0.2 \leq D \leq 3.9$
VI Low-level autoxidized methyl oleate	$-1.0 \leq D \leq 1.6$	$-0.4 \leq D \leq 2.6$

The two values, 92.48 and 91.98%, in Table I, obtained for aliquot 2 on Monday for high purity Tetralin hydroperoxide (I) using the stannous chloride method were obviously not in line with the remaining data in this Latin square. Some erratic behavior or gross experimental error had probably occurred. A supplied mean value of 98.15% was calculated for this position in the block by use of the "missing plot procedure" (2). In all subsequent treatment of these data this calculated value was used instead of 92.23%, the mean of the observed pair of values.

Analyses of variance using the means rather than the two individual values were then applied to the above data for each sample. The results of the analyses of variance are shown in Table II, wherein are represented the mean sum of squares (on the basis of the means of two duplicates) for methods, aliquots, times, interaction, and finally the interactions within the individual methods.

The exceedingly large mean squares (critical at the 1% level) obtained in the comparison of the polarographic vs. the chemical methods for all the samples except Tetralin hydroperoxide (I) were anticipated, as previous work had shown that for impure peroxidic samples the polarographic method tended to give lower results than the Wheeler iodide method. Only in the case of high purity Tetralin hydroperoxide (I) did the values obtained

by the polarographic and chemical methods yield a mean square with a noncritical *F* value at the 5% level. This is consistent with the assumption which is suggested by the means that the chemical methods are measuring substances which are not reducible polarographically and which are presumably not hydroperoxides.

In contrast, the mean squares obtained in the comparison between the Wheeler iodide and the stannous chloride methods show that only in the case of low level autoxidized methyl oleate (VI) was a critical *F* value at the 5% level obtained. This shows that the two chemical methods generally yield very similar results. The noncritical mean squares obtained for aliquots and for times indicates that the subdivision of the sample into aliquots did not significantly affect the peroxide values obtained by the three methods, and further that performing the analyses on three different days of the week had no significant effect. This indicated that the peroxidic samples were relatively stable during the week's period covered by the experiment even though the samples were exposed to atmospheric oxygen in room temperatures and light for several hours on each of the three days of the experiment. At other times they were stored at -5° C. under nitrogen.

The remaining mean squares which appear in Table II under the items entitled interaction within polarographic, within Wheeler iodide, and within stannous chloride did not yield critical *F* values at the 5% level. The mean squares for the within methods interactions were not calculated for high purity Tetralin hydroperoxide because of the replaced value. A further comparison of these mean squares on a probability basis showed that no definite decision could be arrived at concerning which one of the three methods was the more precise.

Table III shows the range of differences between means to be expected at the 5% level for the Wheeler iodide vs. the polarographic and the Wheeler iodide vs. the stannous chloride methods. The range in which the observed difference, *D*, falls shows that the Wheeler iodide method can be expected to give markedly higher values than the polarographic method. Also, the means of duplicates obtained from the stannous chloride method have a tendency to be somewhat higher than those from the Wheeler iodide method.

The table also shows that the means of duplicates obtained using the stannous chloride method had a tendency to be somewhat higher than those obtained by the Wheeler iodide method.

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